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ORIGINAL PAPER

Effects of tallowtree seed coat on seed germination

LI Shu-xian • GU Hong-biao • MAO Yan • YIN Tong-ming • GAO Han-dong

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Abstract: We measured physiological parameters including water uptake, in-vitro embryo germination ratio, and seed coat structure observed by scanning electron microscopy (SEM) to explore the influence of seed coat on the germination of seeds of tallow tree (*Sapium sebiferum* (Linn) Roxb.). Tallow tree seeds had good water permeability. We found that germination of cabbage seeds was inhibited when cabbage seeds were soaked in extracted solutions from tallow tree seed coat. Seed coat structure at the side of the radicle appeared to be a barrier to seed germination. We tested methods to break tallow tree seed dormancy. Dormancy of tallow tree seeds was overcome by soaking the seeds in 500 $mg \cdot L^{-1}$ or 1000 $mg \cdot L^{-1}$ GA_3, followed by 100 days of cold stratification.

Keywords: *Sapium sebiferum*; seed dormancy; cold stratification; inhibitory substances; gibberellic acid

Introduction

Propagation methodologies should be well understood when plants are introduced into the landscape industry. Seeds are typically used for propagating woody plants because this is the most cost-effective method for many species (Rees 1996). During germination, seeds absorb water and swell, eventually resulting in rupture of the seed coat. Finally, the radicle emerges from within the ruptured seed coat. But many seeds are unable to germinate or do so with difficulty even when in apparently favorable conditions. Often, such seeds are considered to be dormant. Seed germination is influenced by factors including seed coat, embryo, inhibitors (Agrawal et al. 1995), or the relative concentrations of hormones (Bewley et al. 1994).

Chinese tallow tree (*Sapium sebiferum* (Linn) Roxb.) of the family Euphorbiaceae is a deciduous tree in most parts of China. It has potential for use as a landscape plant because of its colorful foliage and decorative fruits during autumn. In China, tallow tree is widely planted in gardens and along roadsides (Peng et al. 2010). Tallow tree seeds also have high economic value in industry.

The seed coat of tallow tree is one of the factors inhibiting seed germination. Tallow tree seeds do not germinate rapidly and tidily due to characteristics of their seed coat. This study was designed to determine the influence of seed coat characters on the germination of tallow tree seeds. We investigated the effects of treatments of seed coats in an effort to increase the rate of germination of tallow tree seeds.

Materials and methods

Seed materials

Mature seeds of tallow tree were collected manually on campus of Nanjing Forestry University, Nanjing, China in November 2008. Seeds with white waxed seed coats were excised from the fruits and were stored in a refrigerator at 5 °C. The experiments were conducted by the end of February 2009.

Water uptake experiment

Moisture uptake by seeds was calculated by increment in weight after soaking divided by the initial weight. Three replicates of a sample of about 10 g of seed (containing 45 to 50 seeds) were used for the water uptake experiment. The seeds were immersed in 200-mL distilled water at room temperature for three days. Every eight hours, seeds were removed from the water and immediately surface dried using filter paper. Water absorption rate was calculated as follows:

$$W(\%) = \frac{W_s - W_i}{W_i}\%$$

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LI Shu-xian (✉)• GU Hong-biao • MAO Yan • Yin Tong-ming • GAO Han-dong
College of Forest Resources and Environment, Nanjing Forestry University, Nanjing 210037. E-mail: shuxianli@njfu.com.cn

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where, W is water absorption rate (%); W_s is weight after soaking; W_i is initial weight.

Effect of extracting solutions of seed coat on the germination of cabbage seeds

Tallow tree seed coats were ground into powder and the powder was extracted using a methanol solution. Five grams of powder were extracted 3 times. Each time the powder was dissolved in 100 mL of 80% (v/v) aqueous methanol and then stored for 24 h in a refrigerator at 5°C. After filtration, the combined supernatants were evaporated under vacuum at 37°C to expulse methanol. The aqueous suspensions were adjusted to 0.1 g·mL^{-1} and 0.2 g·mL^{-1}.

We tested the potential of the tallow tree seed coat extract to inhibit germination of cabbage seeds. Germination tests of cabbage seeds were conducted using three replicates. One hundred seeds were soaked in 5 mL of the tallow tree seed coat extract (see paragraph above) at concentrations of 0.1 g·mL^{-1} and 0.2 g·mL^{-1} for 3 h. The control seeds were soaked in distilled water. Seeds were then incubated in lighted environmental chambers maintained at 25°C. Numbers of seedlings were recorded after 7 days. When cotyledons were visible by ISTA rules (International Seed Testing Association 1993), germination percentage of every treatment was calculated.

Germination test of excised mature embryos

Mature seeds of tallow tree were soaked for two days in running water. After excision from their enclosing seed tissues, the embryos were placed in plastic boxes containing wet absorbent cotton at 25°C with an 8-h light period. Germination was monitored every other day for 16 d. Finally the germination percentages of the excised embryos were calculated.

Germination test of stratified tallow tree seeds

Seeds of tallow tree were immersed for 24 h in 400-mL flasks containing 200 mL of three concentrations (200 mg•L$^{-1,}$ 500 mg·L^{-1} and 1000 mg·L^{-1}) of gibberellic acid (GA_3). Seeds soaked in Milli-pore filtered water were used as a control. After soaking, the seeds were spread on trays measuring 40 cm × 30 cm × 10 cm, separated from each other and covered with sterilized sand. Finally they were put in the refrigerator at 0–5 °C for 60 days or 100 days.

Following stratification, seed germination tests were carried out in a growth chamber under optimum conditions (25°C with an 8 h photoperiod) for 30 days. For seeds treated with cold stratification for 60 days, germination was tested for seeds with coats and without coats. Of the seeds treated with 100 days of cold stratification only those with seed coats were tested for germination because the inhibiting effect of seed coat was almost eliminated by 100 days of stratification. Thirty days later, seed germination percentage was determined by the rules for forest tree seed testing (GB2772-1999 2000).

Statistical analysis

The effect of the treatments was assessed by the final germination percentage for each trial. Results from the different treatments were analyzed separately. The significance of treatments was tested by One-Way Analysis of Variance (ANOVA) and the Tukey test was used to identify significant differences between pairs of means.

Seed coat structure by SEM

Seed coat structure of tallow tree was examined by SEM (FEI QUANTA 200). The seeds treated with cold stratification for one and two months were examined, and fresh seeds were used as controls. Examination was conducted as follows: samples of seed coat of 1-2 mm^2 were excised from the seed, fixed in 4% glutaraldehyde, washed in 0.1M phosphate buffer, and dehydrated in a graded series of ethanol solutions (30%, 50%, 70%, 90%, 100%). Finally the samples were replaced with isoamyl acetate. Each treatment was applied for 15 min. Finally the samples were observed and pictured with electron microscopy.

Results

Water uptake

The structure of tallow tree seed coat would inhibit germination by retarding water uptake and also by slowing embryo development (Martins-Loucao et al. 1996).

Water uptake was monitored for 72 h after seeds were soaked in water. Water uptake increased with time (Fig. 1). Water absorption rate was 50.9% at 40 h, after which the rate of water uptake slowed. This experiment showed that the seed coat was permeable and the seed was able to take in water during germination.

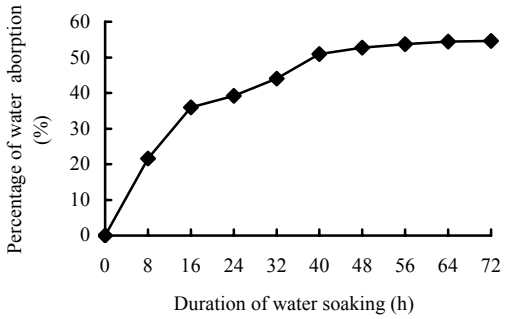

Fig. 1 Water uptake curve of tallow tree seeds

Effects of tallow tree seed coat inhibitor on the germination of cabbage

Many plant seeds contain compounds that inhibit seed germination in other species but cabbage seeds do not. Therefore, cabbage seeds are often used to test the inhibition effect of other

species. The germination percentage of cabbage seeds was 86% when they were not soaked in tallow tree seed coat extract. In contrast, germination was 63% when cabbage seeds were soaked in 0.1-g·mL^{-1} extraction, and 35% when seeds were soaked in 0.2 g·mL^{-1} extraction. Therefore, the inhibitor in tallow tree seed coat was one of the reasons the tallow tree seeds underwent dormancy.

Germination of isolated mature embryo

Tallow tree seeds with seed coats did not germinate at maturity. But embryos excised from mature seeds germinated when subjected to suitable conditions. At 16 days, 80% of tallow tree embryos germinated, demonstrating that the isolated embryos could germinate quickly.

Effects of different GA_3 treatments on germination of tallow tree seed

Data in Table 1 show that germination of untreated tallow tree seeds was lower than that of seeds treated with GA_3. After cold stratification for 100 days, seed germination was 26%, much lower than that of the isolated embryos (80%). This indicates that tallow tree seeds were in deep dormancy.

The seeds of tallow tree, like other seeds, are enclosed by a thick seed coat and we hypothesized that might reduce seed germination. Hulled seeds had significantly higher germination rates than intact seeds (Table 1). This demonstrates that seed coats retarded germination and that seed germination was enhanced by seed coat removal.

Table 1. Germination percentages of tallow tree seeds treated with three concentrations of GA_3 and after 60 days of stratification (intact seed coats and hulled seeds) and after 100 days of stratification (intact seed coats only)

GA_3	Germination (%)		
(mg·L^{-1})	60 d	Hulled coat*, 60 d	100 d
	0±0a	14.3±2.0a	26.0±2.3a
200	16.7±2.3b	32.7±1.7b	65.0±4.0b
500	18.0±3.0b	36.0±3.3b	79.3±3.7b
1000	25.7±3.7b	43.3±3.7b	75.3±3.3b

Notes: *The third column is the treatment that the seeds were hulled coat. Values on the columns followed by the same letter are not significantly different (p<0.05, Tukey test).

The seed germination rates were examined at three GA_3 treatments and two periods of stratification. GA_3 had a significant effect on seed germination when seeds were stratified for 60d or 100d. The promoting effect of GA_3 treatment is often attributed to the mobilization of stored reserves and weakening of the mechanical resistance of the endosperm cells around the radical tip (Tigabu et al. 2001).

The dormancy of tallow tree seeds was only partly overcome by GA_3 treatment. The highest germination percentages of seeds were achieved with treatments of 500 mg·L^{-1} GA_3 combined with cold stratification. Thus the combination of cold stratification and GA_3 improved the germination of tallow tree seeds.

The changes of seed coat structure documented using SEM

Use of SEM revealed changes of seed coat structure due to the treatments. The seed coats on the radical side became thinner on treated seeds than on controls (Fig. 2A). During cold stratification，seed coats became thinner after GA_3 treatment, but remained too thick for seed germination. Dormancy was not completely broken after cold stratification for 60 days (Table 1). Therefore, the seed coats on the radical side inhibited seed germination to some extent.

The morphological changes in the seed coat may also explain in part why seed germination percentage increased while seed stratification time increased. Intercellular spaces in seed coats from different treatments were more abundant than in controls (Fig. 2B), and this was crucial for seed germination because the volume of the aqueous fraction was increased and oxygen could diffuse towards the embryo axis as demonstrated by Lin et al. (2001).

Discussion

Seed coat-imposed dormancy

This research investigated the relationship between tallow tree seed coats and seed germination. The fully excised seed embryos exhibited 80% germination, indicating that there were no germinating inhibitors in the embryos. Our treatments demonstrated that seed coat structure and inhibitor compounds in the coat influence germination of tallow tree seeds. Several germination inhibitors are present in the seed coats of dormant seeds (Bewley et al. 1982). The identity of the inhibitor compound in tallow tree seed coats remains unknown and presents a challenge for future research.

The treatment to overcome seed dormancy

Gibberellins stimulate seed germination of many species of angiosperms and gymnosperms (Leadem 1990; Schmitz et al.2001). Our research confirmed that gibberellins affected the dormancy of tallow tree seeds but did not completely overcome seed dormancy until cold stratification was extended to 100 d. Additional study is needed to optimize procedures to overcome seed dormancy more quickly.

To increase seed germination rates, a variety of seed treatments can be employed. Physical treatments include coat laceration (scarification), coat microfissure (heat and ultrasound), or coat softening and embryo stimulation (pre-chilling and soaking). Chemicals can be used to remove the coat (sulfuric acid), remove the waterproofing or the embryo inhibitors (NaClO and C_2H_5OH), or stimulate the growth of the embryo (GA_3) (Ceccherini et al. 1998). When we treated seeds with concentrated sulphuric acid (H_2SO_4), the inner part of seed was burned to an

extent detectable using SEM (Fig. 3).

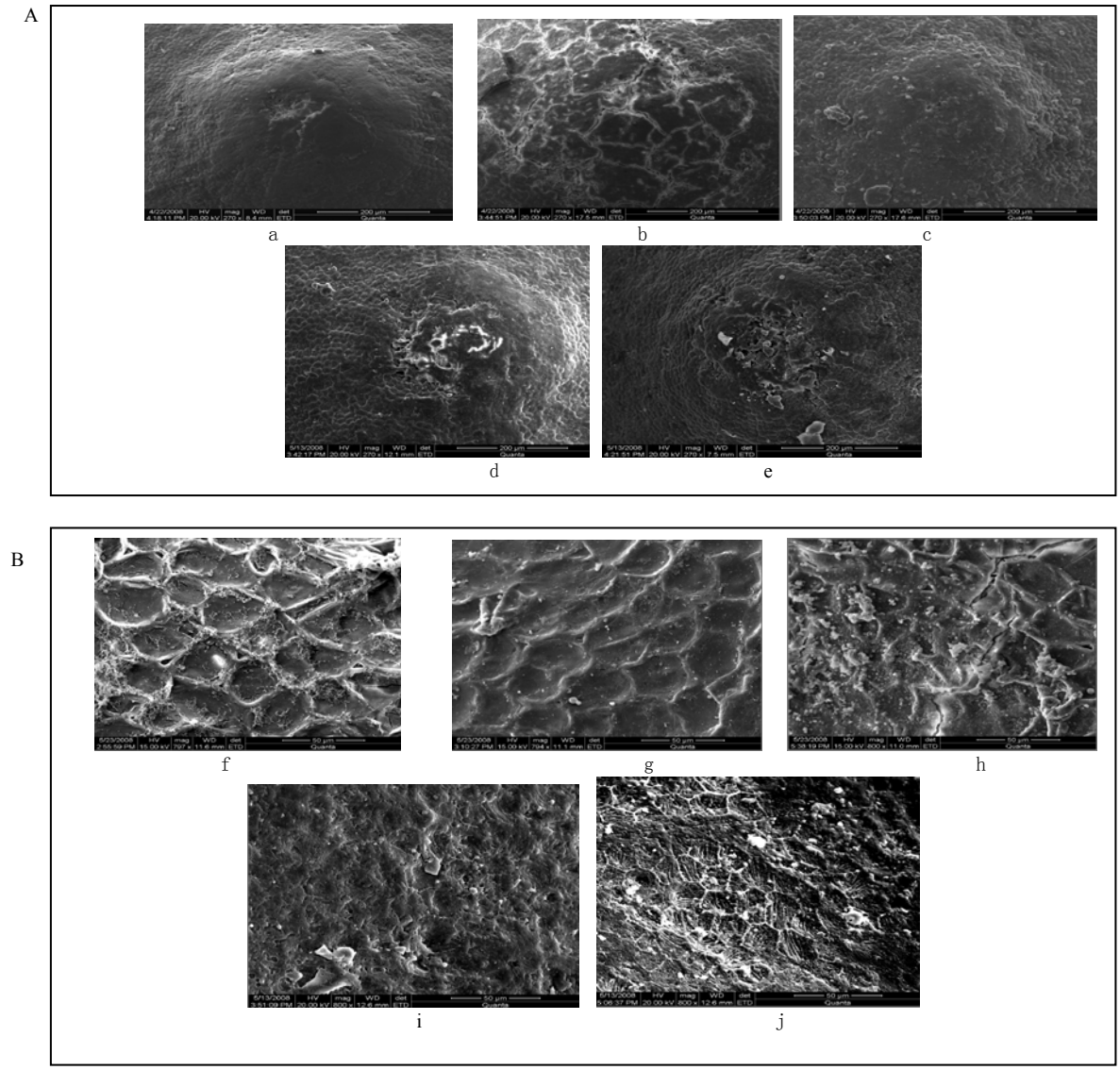

Fig. 2 Changes in tallow tree seed coat structure illustrated by scanning electron microscopy

A is Side near the radical; B is other side of the seed. a is fresh seed; b, cold stratification for 30 d; c, GA_3 (1000 mg·L^{-1})+ stratification for 30 d; d, cold stratification for 60 d; e, GA_3 (1000 mg·L^{-1})+stratification for 60 d; f, fresh seeds; g, cold stratification for 30 d; h, GA_3 (1000 mg·L^{-1})+ stratification for 30 d; I, cold stratification for 60 d; j, GA_3 (1000 mg·L^{-1})+ stratification for 60 d.

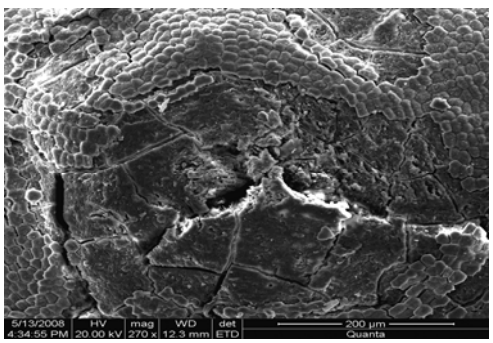

Fig. 3 Scanning electron micrograph shows damage of seed coat after seeds soaked in sulphuric acid for 8 min.

Conclusions

Our results showed that seed coats inhibited germination of tallow tree seeds. Our experiments revealed that seed coat structure and the inhibitory compound(s) in the seed coats played important roles in causing the dormancy of tallow tree seeds. We found the seed coat to be thicker at the radical side than on other parts of the seed coat and we hypothesize that this thickening was a major barrier to seed germination. We tested various treatments to break seed dormancy. Our results showed that seeds must be treated to remove the physical or chemical barrier to germination. Cold stratification combined with gibberellins (GA_s) significantly improved seed germination. Among the different methods

tested in this study, the most effective treatment was soaking seeds in 500 or 1000 mg·L^{-1} GA_3, following cold stratification for 100 days.

Acknowledgements

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